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Abbe has shown that by transmitted light diffraction images are obtained and refraction images by reflected light. Not to enter into a discussion of these different images it suffices to say that the author had spent hours at a time trying to catch a glimpse of the striae and invariably failed, until one day a lucky accident brought them to view. On this occasion while working near a window through which the direct sunlight happened to strike the top of the stage of the microscope, a sudden accidental movement so placed the stand as to exclude the light from the mirror. The direct sunlight fell on the edges of the drop of immersion oil and immediately the markings came out clearly, sharply, and unmistakably. After a certain amount of experimentation it was found that the best results were obtained when the incidence of the direct sunlight took place as nearly as possible parallel to the stage, the microscope being inclined for the purpose, and at right angles to the striae. From this it would seem that the lines are alternate ridges and depressions on the diatom shell and the lines were brought out as contrasts of ridges with their shadows. The method is so easy and simple that the merest beginner in microscopy could bring out the markings without any difficulty. The method has been used with great advantage in our laboratories in determining the markings on the spores of the Myxomycetes, as this characteristic is at times an important factor in the determination of these plants.

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## Notes on Histological Technique.

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### I. A SIMPLIFIED MODIFICATION IN A STAINING METHOD.

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#### J. HUERKAMP.

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The usual method of staining plant tissues with Delafield's haemtoxylin and safranin as described in the common texts of histology and microtechnique, requires that the preparation remain in the latter stain about 12 hours in order that sufficient and satisfactory penetration of the color may result. Tissues of certain plants will take the stain much more rapidly and retain it more tenaciously, while others even after being kept in the stain longer than is ordinarily required can not be made to keep it at all.

It is well known that saffranine, like many of the so-called aniline dyes, is intensified in the presence of alkalies, and weakened or entirely removed in the presence of acids. Advantage is made of this fact in the following modification of the method used in our laboratory for a long time. The time needed for the preparation of slides is very much shortened and the results are quite as satisfactory in regard to the quality of the microscopical slides.

Sections of plant tissue to be double-stained with Delafield's haematoxylin and saffranine after being attached to the slide with albumen fixative, are slightly warmed and the paraffine removed with xylol, and the xylol, with alcohol in the usual way. The alcohol is removed by immersion in water, and the preparation over-stained with the haematoxylin. Excess of stain as also the cloudiness and muddiness is removed by washing for a second or more as is found necessary, in dilute hydrochloric acid.

When the proper reduction of the color has been obtained the acid is rapidly removed by washing in water, and the slide may immediately be stained with saffranine, which need not be allowed to act more than a minute or two, and often less. Penetration of the color is further insured by putting the slide in ordinary tap-water which removes the excess of stain and fixes it into the preparation, because of the traces of alkali usually present in ordinary water. Distilled water is therefore undesirable in washing off stains. After removal from the water, which may be done almost immediately, the preparation will be found to be over-stained, and the excess of red color may be taken out by washing with 95 per cent alcohol to which a small amount of slaked lime has been added. This alkali is but very slightly soluble in alcohol,\* yet sufficiently so to cause the further fixation of the red in the desirable parts of the tissue, and the removal of the same from the other parts already stained with the haematoxylin. Wood tissue and nucleus will take up the saffranine, and the cellulose remain blue or purple-blue with the haematoxylin. If the red does not come out as quickly as desired, alcohol without any alkali may be used in washing.

The preparation of microscopic slides by the process described is so rapid that the slides may be made by the dozen at a time, in a few minutes, and the preparation of a single slide does not take much longer than it does to describe the above method. If saffranine is used before the haematoxylin the results do not seem to be as easily obtained as by the procedure outlined.

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\* About 3 milligrams of calcium hydroxide of slaked lime has, by experiment been found to dissolve in 100 cc. of absolute alcohol, or less than 1 gram in 30,000 parts by volume of alcohol. The solubility increases, however, in proportion to the general amount of water present, but in 95 per cent alcohol it is still very small.